Long-term soil microbial community and enzyme activity responses to an integrated cropping-livestock system in a semi-arid region ☆

V. Acosta-Martínez [a,*], C.W. Bell [b], B.E.L. Morris [c], J. Zak [b], V.G. Allen [d]

[a] USDA-ARS, Cropping Systems Research Laboratory, 3810 4th Street, Lubbock, TX 79415, United States
[b] Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409, United States
[c] Department of Botany & Microbiology, Institute for Energy & The Environment, The University of Oklahoma, 770 Van Vleet Oval, Norman, OK 73019-0245, United States
[d] Department of Plant and Soil Science, Texas Tech University, Lubbock, TX 79409, United States

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ABSTRACT

Water availability is a primary limiting factor facing agricultural systems in most semi-arid regions across the world. This study is part of a larger long-term project to develop and evaluate integrated crop and livestock systems in order to reduce dependence on underground water sources by optimizing cotton (*Gossypium hirsutum*) production in the Texas High Plains of U.S. Selected microbial, chemical and biochemical properties were studied (between 7 and 10 years) in a clay loam soil (fine, mixed, thermic Torrertic Paleustolls) under continuous cotton compared to an integrated cropping-livestock system that included cotton, forage, and Angus-cross-stocker beef steers (initial body weight 249 kg). For the integrated system, steers grazed in sequence a perennial warm-season grass 'WW-B. Dahl' old world bluestem (*Bothriochloa bladhii*) paddock, and then rye (*Secale cereale* L.) and wheat (*Triticum aestivum* L.) grown in two paddocks (stages) of a rotation with cotton. Our previous studies after 5 years showed greater microbial biomass C (MBC) in perennial pasture (193 mg kg^{-1} soil) and the rotation when sampled under rye or cotton (average of 237 mg kg^{-1} soil) compared to continuous cotton (124 mg kg^{-1} soil) at 0–5 cm. After 7 years, MBC became significantly higher in the rotation independent of the crop compared to continuous cotton in this study. At the end of 10 years, total C was higher in both the rotation and pasture of the integrated cropping-livestock system (average across grazing treatments: 17.3 g kg^{-1} soil) compared to continuous cotton (11.4 g C kg^{-1} soil). Soil MBC and several enzyme activities were higher under non-grazed areas compared to grazed areas within the integrated cropping-livestock system in some samplings. Microbial community structure of pasture soil showed higher FAME indicators for G− (i.e., *a*17:0 and cy19:0) and actinomycetes (i.e., 10Me17:0) under grazed areas compared to non-grazed areas. Microbial community structure of pasture soil showed higher fungal populations compared to continuous cotton. The rotation showed intermediate sum of bacterial FAME indicators among systems (continuous cotton > rotation > pasture) and a tendency for numerically slightly higher fungi:bacterial ratios compared to continuous cotton. This study demonstrated increases in microbial biomass and enzyme activities of C-, N-, P- and S-cycling within an integrated cropping-livestock system that may represent positive changes in soil functioning compared to continuous cotton.

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1. Introduction

Water availability is a significant factor limiting agricultural production in many semi-arid regions including the Texas High Plains region. Efforts to sustain cotton production in this region have focused on integrating small grain crop rotations with cotton and pasture for livestock production systems in order to reduce irrigation needs relative to typical continuous cotton monoculture (TAWC, 2007; Allen et al., 2005, 2008). The benefits for integrated livestock-crop production systems compared to specialized systems (i.e., monoculture crop production) has been shown to reduce water usage and fuel costs associated with irrigation, and to improve soil chemical, microbial and/or physical properties (Franzluebbers, 2007) with potential positive implications to soil quality (Acosta-Martínez et al., 2004).

To evaluate potential soil quality enhancements including soil nutrient cycling capabilities, soil C sequestration and other processes, the size, composition and activity of soil microbial communities must be considered. Soil microbial communities, as the primary source of soil enzymes, mediate critical nutrient cycling

functions such as decomposition, nutrient mineralization, and soil organic matter transformation (Tabatabai, 1994; Sotomayor-Ramirez et al., 2009). Furthermore, the soil microbial component is sensitive to changes in soil due to land use or management before changes in other soil properties are detected (Gregorich et al., 2006; Acosta-Martínez et al., 2007). Ingram et al. (2008) stated that to better understand C- and N-cycling, it is important to gain understanding of microbial communities and related processes. Further, studying several enzyme activities involved in C, N, P and S cycling can provide information of soil metabolic or functional responses to changes in management practices (Powlson et al., 1987; Turco et al., 1994; Kandeler et al., 1999; Kennedy, 1999; Karlen et al., 2003).

In 1997, a long-term research site was established in the Texas High Plains in order to compare a monoculture cotton cropping system with an alternative system that integrated cotton and livestock production, in which cattle sequentially grazed a 2-paddock cotton and small grain crop rotation and a perennial pasture paddock throughout the year while maintaining cotton production during summer. This type of cattle grazing is a common practice in the livestock production industry sector, which views the rotation and pasture as a single integrated cropping-livestock system because of the interaction of the sequential cattle grazing between pasture and crop-rotation paddocks over time. Non-grazed zones were also established within each paddock area to evaluate long-term soil responses to the effects of grazing within the integrated cropping-livestock system.

To evaluate the sustainability of the integrated cropping-livestock system agriculture practices, many different research activities were conducted at this Texas High Plains long-term research site. Research during the first 5 years (1997–2002) only assessed soil responses to the integrated cropping-livestock system in the non-grazed areas, and demonstrated that a tilled monoculture cotton system required more irrigation, was more susceptible to wind-induced soil erosion, and showed lower soil organic matter and microbial biomass than in the integrated cropping-livestock system (Acosta-Martínez et al., 2004; Allen et al., 2005, 2008). Furthermore, soil (0–5 cm) under the perennial grass pasture demonstrated higher soil organic C, aggregate stability, microbial biomass C, enzyme activities, and protozoan and fungal abundances when compared to continuous monoculture cotton; however, this trend for the two-paddock rotation within the integrated cropping-livestock system was crop dependent when compared to continuous cotton during the first 5 years of the study (Acosta-Martínez et al., 2004). Thus, it is important to evaluate beyond 5 years the responses of soil microbial communities to this rotation compared to continuous cotton. In addition, it is important to evaluate the grazing effects on the soil microbial communities and functionality within the integrated cropping-livestock system which have not been addressed. Relatively little is known about the long-term impacts of grazing on soil microbial communities and metabolic functioning. Research in grazed systems have shown contradictory responses of the soil microbial communities as many factors likely contribute to grazing effects on soil, including inherent soil properties, soil interactions with vegetation, environmental factors, and intensity of grazing (Schuman et al., 1999; Parton et al., 2001; Ganjegunte et al., 2005).

This study intends to evaluate the long-term (10 years) sustainability of integrated cropping-livestock practices at this Texas High Plains research site. Our first objective is to elucidate the soil microbial community size (microbial biomass C and N), structure (microbial functional groups abundances using fatty acid methyl ester profiling) and function (enzyme activities of C, N, P and S cycling) as indicators of potential soil quality changes between these contrasting management practices between 7 and 10 years. The second objective is to evaluate the long-term grazing effects (grazed vs. non-grazed areas) on the microbial community

size, structure and functionality within the integrated cropping-livestock system. Therefore, this 10-year study will allow for a more complete and realistic assessment of the integration of livestock and cotton production impacts, and it will include grazing effects which were not investigated in first 5 years of this study. We hypothesize that a lag time greater than 5 years occurs before differences in soil microbial responses between integrated cropping-livestock system when compared to continuous cotton. Thus, this study intends to resolve if the continuation of an integrated livestock-cotton system beyond 5 years can cause a shift in microbial dynamics in the crop rotation compared to continuous cotton. This finding would suggest that an integrated cropping-livestock system as a whole is a viable option to maintain the sustainability of cotton-farming practices in semi-arid regions of the Texas High Plains by promoting soil quality and ecosystem health. We contend that soil microbial community structural and functional components between differing long-term cropping management practices (monoculture vs. integrated livestock-cotton system) will not be similar. We hypothesize that soil microbial biomass will maintain the highest levels in the pasture and lowest in the continuous cotton, with intermediate levels in the two-paddock crop rotation areas. Furthermore, very different microbial functional group abundances and enzyme activity levels in the soils should be apparent among these cropping management practices.

2. Materials and methods

2.1. Experimental design and soil management

The research site was initiated in 1997 at the Texas Tech University field laboratory located in northeast Lubbock County in the Texas High Plains (101°47′ west longitude; 33°45′ north latitude; 993 m elevation). This area has a dry steppe climate with mild winters. Mean annual precipitation is 465 mm (with most of the precipitation occurring from April through October). The soil was Pullman clay (Fine, mixed, thermic Torrertic Paleustolls) with an average pH of 7.4, 38% clay, 28% silt and 34% sand. A more detailed description of this research site can be found in Allen et al. (2005) and Acosta-Martínez et al. (2004) where soil microbial properties at year 5 were previously published. In brief, the research site was established to compare a continuous cotton system and an integrated cropping-livestock system, which were replicated three times in a randomized block design for a total of 12.75 ha in the experimental area (Fig. 1). Generally, both systems were irrigated with an underground drip irrigation system with tapes located on 1-m centers and buried about 0.36 m deep. The cotton varieties for both systems were Paymaster 2326RR (1998–2002), 'Fibermax 989 BR03-04 (2003–2004), Fibermax 960BR05 (2005) and Fibermax 9058F (2007).

Each replication of the continuous cotton system was comprised of a single 0.25-ha plot. Cattle were not a part of this system. Nitrogen was applied through the irrigation tape during the growing season (mean N application rate was 150 kg ha^{-1}). Phosphorus, K, and S were the only other supplemental nutrients applied as recommended by post-planting soil nutrient analysis. In addition, chemicals, herbicides, and plant growth regulators were applied as recommended by integrated pest management specialists.

Each replicate of the integrated cropping-livestock system comprised 4 ha divided into three- paddocks. Approximately 53.6% (2.14 ha) of the area consisted of a perennial warm-season grass 'WW-B.-Dahl' old world bluestem [*Bothriochloa bladhii* (Retz) S.T. Blake] paddock. The remaining 46.4% of the system was divided equally into 2-paddocks for two stages of a small grain forage–cotton rotation (0.93 ha each). Livestock production was
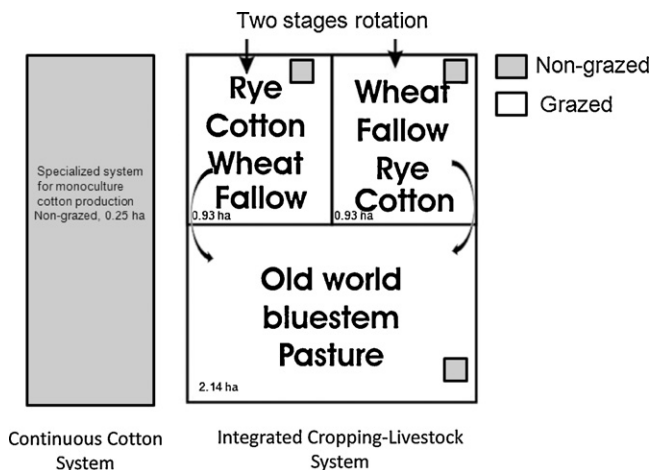
Fig. 1. Drawing representation of the systems compared at the Texas Tech University field research site: continuous cotton system and an integrated cropping-livestock system. In the integrated system, livestock grazed in sequence a pasture paddock and two paddocks with different stages of the same rotation, except for non-grazed zones within each paddock excluded from grazing throughout the study. Cattle were not part of the continuous cotton system. This figure intends to describe the systems studied using one of the three field replicates only, and does not intend to represent the exact size and location of systems or cages in the field.

maintained throughout the whole year as Angus and Angus-cross-beef steers (*Bos taurus*) sequentially grazed the pasture and the forages available in the (two stages) rotation while cotton was produced in one of the two stages of the rotation in summer. Specifically, 'Maton' rye (*Secale cereal* L.) was planted in early September and subsequently grazed by steers in sequence with dormant stock-piled old world bluestem from January until mid- to late-April. Following grazing, rye was terminated with glyphosate, and cotton was planted (no-till) into rye residue. After the cotton was harvested in late autumn, 'Lockett' wheat (*Triticum aestivum* L.) was planted (no-till) into cotton residue. Wheat was grazed throughout the following spring until a second old world bluestem grazing interval was initiated, which lasted until mid-July. After July, all steers were removed from the system, and land was fallowed until rye was planted the following September. The stage of the rotation under fallow was sprayed with glyphosate to minimize weeds and clipped (at least once) to eliminate volunteer cotton. About 80 kg ha^{-1} N was applied once each spring to the old world bluestem pastures. Rye and wheat each were fertilized with about 67 kg N ha^{-1} each year. Within each paddock, caged grazing exclusions (4.8 m × 4.8 m) were established at the beginning of this system and remain intact in the same location during the study. These grazing exclusions allow studies to determine the effects of grazing vs. no grazing. Forage within the caged areas were managed as a hay crop. The caged areas were otherwise consistently managed like the grazed areas. Cages were removed for planting and harvesting at the end of each grazing sequence, and were then reinstalled prior to the next grazing season.

2.2. Soil sampling

The sampling approach was described by Acosta-Martínez et al. (2004) for soil samples (0–5 cm) taken in fall 2002 (November) and summer 2003 (July) from the integrated cropping-livestock and continuous cotton systems. Similarly, the soil samples for this study were taken in summer 2004 (June), fall 2006 (November) and summer 2007 (July). In brief, one (composite) sample was taken from each field replicate of the treatments (continuous cotton, each of the two stages rotation, and pasture). Generally for the rotation, samples were taken from both cotton and fallow periods in summer

(2004 and 2007) and both wheat and rye in fall (2006) due to the availability of two stages of the rotation in the same year as shown in Fig. 1. In order to compare the effects of grazing on the soil microbial component within the integrated cropping-livestock system, soil samples were taken from grazed and non-grazed cage areas. Soil pH was determined in the air-dried soil (<5 mm) using a combination glass electrode (soil:water ratio, 1:2.5). Total C and total N were determined in air-dried soil (<180 μm) in a private laboratory (Ward Laboratories, Nebraska) by automated dry combustion (LECO TruSpec CN; Joseph, MI).

2.3. Soil analyses

2.3.1. Microbial biomass

The MBC was determined on a 15-g oven-dry equivalent field-moist soil sample (<5 mm) by the chloroform-fumigation-extraction method using 0.5 M K_2SO_4 as an extractant (Vance et al., 1987). In brief, organic C from the fumigated (24 h) and non-fumigated (control) soil were quantified by a CN analyzer (Shimadzu Model TOC-V/$_{CPH}$-TN). The non-fumigated control values were subtracted from the fumigated values. The MBC was calculated using a k_{EC} factor of 0.45 (Wu et al., 1990). Each sample had duplicate analyses and results are expressed on a moisture-free basis. Soil moisture was determined after drying at 105 °C for 48 h.

2.3.2. Microbial community structure with FAME analysis

FAME analysis was conducted following the MIDI (Microbial ID, Inc., Newark, DE, USA) protocol as previously applied to soil analyses (Acosta-Martínez et al., 2004). In brief, the four steps of the MIDI protocol applied on the field-moist soil samples (3 g) follows: (1) saponification of fatty acids at 100 °C with 3 ml 3.75 M NaOH in aqueous methanol [methanol: water ratio = 1:1] for 30 min; (2) methylation (esterification) at 80 °C in 6 ml of 6 M HCl in aqueous methanol [1:0.85] for 10 min; (3) extraction of the FAMEs with 3 ml of 1:1 [v/v] methyl-tert-butyl ether/hexane; and (4) washing of the solvent extract with 1.2% [w/v] NaOH. The FAMEs were analyzed in a 6890 GC Series II (Hewlett Packard, Wilmington, DE, USA) equipped with a flame ionization detector and a fused silica capillary column (25 m × 0.2 mm) using H2 (ultra high purity) as the carrier gas. The temperature program was ramped from 170 °C to 250 °C at 5 °C min^{-1}. Fatty acids were identified and quantified by comparison of retention times and peak areas to components of MIDI standards. The FAMEs relative peak areas (percentage) were determined with respect to the other FAMEs in a sample using the Aerobe method of the MIDI system. Individual peak data for each fatty acid were converted to molar percentages by dividing peak area by the fatty acid molecular weight, then dividing by the total molar area of all fatty acids identified in the sample (Liebig et al., 2006). The FAMEs are described by the number of C atoms, followed by a colon, the number of double bonds and then by the position of the first double bond from the methyl (ω) end of molecules. Cis isomers are indicated by c, and branched fatty acids are indicated by the prefixes *i* and *a* for iso and anteiso, respectively. Other notations are Me for methyl, OH for hydroxy and cy for cyclopropane.

2.3.3. Enzyme assays

The activities of acid phosphatase, alkaline phosphatase, phosphodiesterase, arylsulfatase, α-galactosidase, and β-glucosidase were assayed using 1 g of air-dried soil (<5 mm) with their appropriate substrate (*p*-nitrophenyl derivate) and incubated (37 °C) at their optimal pH as described in Tabatabai (1994). Similarly, β-glucosaminidase activity was determined as described by Parham and Deng (2000). The results for this group of enzyme activities were expressed in mg of *p*-nitrophenol (PN) released kg^{-1} soil h^{-1}. Three amidohydrolases (amidase, urease, and L-asparaginase) were assayed as also described in Tabatabai (1994), and the results were
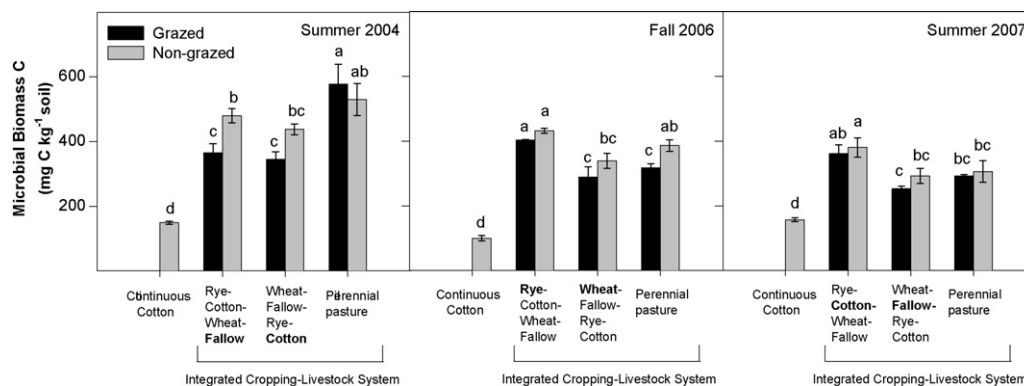
Fig. 2. Microbial biomass C as affected by the system (integrated crop-livestock system vs. continuous cotton) and grazing treatment (grazed vs. non-grazed areas) at 0–5 cm. Similar letter at the same depth indicates no significant treatment differences according to least significant differences (LSD) at $P < 0.05$. MBC values are expressed as mean ± S.E.

expressed in NH_4^+–N released g^{-1} soil h^{-1}. The steam distillation for the assay of amidohydrolases was performed using a Foss Kjel-tec 2200 Auto Distillation Unit (FOSS North America, Eden Prairie, MN), and the distillate was titrated using a Mettler Toledo DL 50 titrator (Mettler-Toledo Inc., Columbus, OH). All enzyme activities were assayed in duplicate with one control, to which substrate was added after incubation and subtracted from a sample value.

2.4. Statistical analyses

The data were analyzed as a randomized block design model in SAS (version 9.1) for the summer 2004, fall 2006, and summer 2007 sampling periods evaluated in this study. Analysis of variance was performed to determine system effects (continuous cotton vs. integrated cropping-livestock system under non-grazed areas) and grazing effects within the integrated cropping-livestock system. Contrast comparisons were calculated to determine grazing effects for pasture and the rotation using Tukey adjustments. Least significant differences were calculated at $P \leq 0.05$ for graph plots to compare the soil microbial properties among continuous cotton, the rotation under different crops, and pasture. Throughout this manuscript, the crop being produced for the rotation at the time of sampling is indicated in bold. Principal component analyses (PCAs) were performed using the PCORD program (version 5) with FAME indicators of bacterial (G+, G−, and actinomycetes) and fungal groups in order to compare the soil microbial community in continuous cotton and the integrated cropping-livestock system. In the PCA, square root transformations were performed on all FAMEs to create a more normally distributed data set and to reduce the coefficient of variation among FAMEs. The square root transformation is similar to log transformations, which is commonly used in ecological studies (McCune and Mefford, 1999). The PCAs were performed using cross-products matrix with variance/covariance centered and calculating scores for FAMEs by weighted averaging. The relative abundance of fungal FAMEs was investigated with the sum of 18:1ω9c, 16:1ω5c, 18:2ω6c and 18:3ω6c. Bacterial populations were studied using indicator FAMEs for G+ (a15:0, i15:0, a17:0, and i17:0), G− (cy17:0, cy19:0, i13:0 3OH, and i17:0 3OH) and actinomycetes (10Me16:0, 10Me17:0). The FAMEs used have been previously suggested as for soil microbial groups in soil analysis (Wright, 1983; Frostegård et al., 1993; Zelles, 1997; Olsson, 1999; Madan et al., 2002). The PCA plots were reproduced in Sigma Plot (ver. 11).

Another set of PCAs were performed in the PCORD program for ten soil enzymes activities to compare the soil metabolic capacity or diversity in the continuous cotton and integrated cropping-livestock systems. The enzyme activities are

involved in C-cycling (β-glucosidase, α-galactosidase, and β-glucosaminidase), N-cycling (β-glucosaminidase, amidase, urease, and L-asparaginase), P-cycling (acid phosphatase, alkaline phosphatase, and phosphodiesterase) and S-cycling (arylsulfatase). These PCAs were also reproduced in Sigma Plot (ver. 11).

3. Results

3.1. Selected soil properties and microbial community size

Soil pH remained relatively constant and was not affected by management throughout the 10-year study (Table 1). Soil moisture content did not show a particular trend among systems. Generally, soil total C and total N showed similar trends as MBC, which differed between the integrated cropping-livestock system and continuous cotton system according to ANOVA (Table 2). The soil MBC levels were higher in pasture (almost 3 times) and within the crop rotation despite the crop (>2 times) when compared to continuous cotton (Fig. 2).

Within the integrated cropping-livestock system, contrast comparisons showed significant grazing effects for MBC under pasture in fall 2006, and for the rotation in summer 2004 (Table 2). In samplings where grazing effects were detected, MBC was higher under non-grazed areas compared to the grazed areas (Fig. 2). Grazing effects were not significant for soil total C and soil pH while were significant (higher under non-grazed areas) for soil moisture and total N in some sampling times (Tables 1 and 2).

3.2. Soil microbial community structure

The individual FAME indicators for bacteria (G+, G−, actinomycetes) and fungal populations responded to the integrated cropping-livestock system compared to the continuous cotton system ($P < 0.05$) across sampling periods (Table 3). The PCAs further supported these findings, as the PCA plots displayed the same separation among the systems with respect to microbial community structure regardless of sampling period (Fig. 3). PCAs showed a clear separation of soil microbial community structure between grazed and non-grazed areas of pasture. For summer 2004, soil pasture showed significantly higher G− indicators (specifically a17:0 and cy19:0) and lower actinomycetes (i.e., 10Me17:0) in the non-grazed areas (Table 3). In addition, the different soil microbial community under the rotation compared to continuous cotton was due to lower G−, actinomycetes and overall sum of bacterial populations. The PCA for summer 2007 showed (along PC1) a different soil microbial community in pasture under grazed areas compared to non-grazed areas due to higher sum of G− indi-

Table 1
Selected soil properties (0–5 cm) under the integrated cropping-livestock system compared to continuous cotton.

Soil property	Integrated cropping-livestock system						Continuous cotton
	Perennial pasture		Rye–cotton–wheat–fallow[a]		Wheat–fallow–rye–cotton		
	Grazed	Non-grazed	Grazed	Non-grazed	Grazed	Non-grazed	
Summer 2004							
Total C (g kg^{-1} soil)	13.34b[b]	13.92ab	16.34a	13.15b	12.57bc	13.73ab	11.82c
Total N (g kg^{-1} soil)	–	–	–	–	–	–	–
pH	7.43a	7.67a	7.47a	7.52a	7.53a	7.48a	7.70a
Moisture (%)	17.5c	16.7c	17.8c	25.1a	12.6d	16.3c	19.9b
Fall 2006							
Total C (g kg^{-1} soil)	15.27b	18.95a	15.27b	17.21ab	17.21ab	16.82ab	10.83c
Total N (g kg^{-1} soil)	1.48a	1.81a	1.46a	1.81a	1.72a	1.69a	1.09b
pH	7.53a	7.55a	7.48a	7.23a	7.39a	7.39a	7.69a
Moisture (%)	14.3ab	16.3a	10.4ab	13.5ab	10.7ab	16.4a	9.1b
Summer 2007							
Total C (g kg^{-1} soil)	16.24ab	17.7ab	14.89b	17.40ab	19.53a	18.17ab	11.41c
Total N (g kg^{-1} soil)	1.30b	1.46ab	1.55ab	1.71a	1.68a	1.58ab	1.07c
pH	7.47a	7.46a	7.18a	7.20a	7.34a	7.42a	7.68a
Moisture (%)	14.2bc	13.9c	17.4a	19.6a	16.6ab	17.3ab	16.8ab

[a] Soil samples were collected under different crops in the rotation in summer 2004 or 2007 (wheat–fallow–rye–cotton or rye–cotton–wheat–fallow) vs. fall 2006 (wheat–fallow–rye–cotton or rye–cotton–wheat–fallow). Please refer to Fig. 4, where the crop being produced for the rotation at each sampling time is indicated in bold.
[b] Different letter indicate significant differences among cropping systems each year from LSD's at $P < 0.05$.

cators (significant for a17:0 and cy19:0) and significantly lower sum of G+ indicators (i.e., i15:0, a15:0, and a17:0) in the grazed areas. Furthermore, the rotation (grazed and non-grazed) showed significant differences in the soil microbial community structure when sampled under fallow (rye–cotton–wheat–fallow) compared to samples collected under cotton (wheat–fallow–rye–cotton) due to higher i17:0, a17:0, cy19:0, 10Me16:0 and 10Me17:0 when it was sampled under fallow periods. The PCA for fall 2006 showed a significantly different microbial community structure in pasture under grazed areas compared to the non-grazed areas, and significant differences in the microbial community structure of the rotation compared to continuous cotton with no grazing effects for the rotation.

3.3. Soil metabolic functioning indicated by enzyme activities

Among 10 soil enzyme activities evaluated for each sampling time, results for selected enzyme activities (β-glucosaminidase, β-glucosidase and alkaline phosphatase) are presented in Fig. 4 and Table 2. The predominant trend found in this study was the higher ($P < 0.01$) enzyme activities under the integrated cropping-livestock system (pasture and the rotation) compared to the continuous cotton system as with microbial biomass C data. Fig. 4 also shows that grazing effects within the integrated cropping-livestock system were found for certain enzyme activities. For example, β-glucosaminidase activity was higher under the non-grazed areas compared to the grazed areas in pasture soil in

Table 2
ANOVA results (P values) to determine system effects (integrated cropping-livestock vs. continuous cotton), grazing effects (non-grazed vs. grazed areas) within the integrated cropping-livestock system, and contrast comparisons for individual treatments (i.e., rotation under different crops, grazing in rotation or pasture).

Comparisons	MBC	Total C	Total N	Selected enzyme activities		
				Alk. phosph act.	β-Glucosidase act.	β-Glucosidaminidase act.
Summer 2004						
System effects (cont. cotton vs. integrated system)	0.01	0.01	–	0.01	0.01	0.001
Integrated cropping-livestock system (pasture vs. rotation)	0.05	n.s.		n.s.	n.s.	0.010
Grazing effects (grazed vs. non-grazed for integrated system)	n.s.	n.s.		n.s.	n.s.	0.05
Wheat–fallow–rye–**cotton**	0.01	n.s.	–	n.s.	0.05	n.s.
Rye–cotton–wheat–**fallow**	0.01	n.s.	–	0.05	0.05	n.s.
Pasture	n.s.	0.01	–	n.s.	n.s.	0.001
Grazing × integrated cropping-livestock system	n.s.	n.s.		n.s.	0.01	n.s.
Rotation sampled under cotton vs. fallow	n.s.	n.s.	–	n.s.	n.s.	n.s.
Fall 2006						
System effects (cont. cotton vs. integrated system)	0.0001	0.01	0.05	0.001	0.01	0.01
Integrated cropping-livestock system (pasture vs. rotation)	0.05	n.s.	n.s.	0.01	n.s.	0.05
Grazing effects (grazed vs. non-grazed for integrated system)	0.01	0.10	0.01	0.1	n.s.	n.s.
Rye–cotton–wheat–fallow	n.s.	0.05	0.05	0.1	n.s.	n.s.
Wheat–fallow–rye–cotton	n.s.	0.05	0.1	n.s.	n.s.	n.s.
Pasture	0.1	0.01	0.05	0.01	n.s.	0.01
Grazing × integrated cropping-livestock system	n.s.	n.s.	0.1	0.05	n.s.	n.s.
Rotation sampled under rye vs. wheat	0.01	n.s.	n.s.	0.1	n.s.	n.s.
Summer 2007						
System effects (cont. cotton vs. integrated system)	0.01	0.05	0.05	0.001	0.01	0.01
Integrated cropping-livestock system (pasture vs. rotation)	n.s.	n.s.	n.s.	0.1	0.01	0.001
Grazing effects (grazed vs. non-grazed for integrated system)	0.1	n.s.	n.s.	0.01	n.s.	0.10
Rye–**cotton**–wheat–fallow	n.s.	n.s.	0.05	n.s.	n.s.	n.s.
Wheat–**fallow**–rye–cotton	0.10	0.05	0.10	0.1	n.s.	n.s.
Pasture	n.s.	n.s.	n.s.	0.05	0.05	0.01
Grazing × integrated cropping-livestock system	n.s.	n.s.	n.s.	0.05	n.s.	n.s.
Rotation sampled under cotton vs. fallow	n.s.	n.s.	n.s.	0.05	0.05	n.s.

Table 3
Comparison of microbial groups in the integrated cropping-livestock system (rotation and pasture) under grazed and non-grazed areas compared to the continuous cotton system[a].

System	G+		G−		Actino		Bacterial sum[b]		Fungal sum[c]		Fungi: bacteria ratio	
	Grazed	Non-grazed	Grazed	Non-grazed	Grazed	Non-grazed	Grazed	Non-grazed	Grazed	Non-grazed	Grazed	Non-grazed
Summer 2004												
Cont. cotton	−[d]	9.375a	−	3.077a	−	3.506a	−	12.452a	−	22.271c	−	1.825c
Rye–cotton–wheat–**fallow**	8.304ab	8.534a	1.753b	1.671b	2.424b	0.904e	10.057b	10.205b	28.692bc	24.610bc	2.945bc	2.500bc
Wheat–fallow–rye–**cotton**	8.719a	7.075b	1.461b	1.805b	1.187de	2.177bc	10.180b	8.880b	21.410c	28.765b	2.077bc	3.177b
Pasture	5.395c	5.695c	1.588b	1.053c	1.753cd	2.433b	6.983c	6.748c	38.060a	40.661a	5.739a	6.283a
Fall 2006												
Cont. cotton	−	9.528a	−	2.937a	−	2.683a	−	12.465a	−	20.681b	−	1.671b
Rye–cotton–wheat–fallow	8.031a	8.653a	1.506b	1.873b	2.024a	0.734c	9.537b	10.527ab	20.380b	21.267b	2.141b	2.037b
Wheat–fallow–rye–cotton	8.751a	9.396a	1.951b	2.132b	1.331b	0.932bc	10.702ab	11.528ab	20.754b	21.431b	1.948b	1.939b
Pasture	4.002b	4.726b	0.858c	0.965c	1.360b	1.934a	4.860c	5.691c	40.660a	43.972a	9.059a	10.147a
Summer 2007												
Cont. cotton	−	11.415a	−	2.446a	−	1.710b	−	13.861a	−	17.209b	−	1.244c
Rye–**cotton**–wheat–fallow	10.543a	11.320a	1.476bc	1.615b	0.301c	3.834a	12.019b	12.936ab	21.676b	18.731b	1.809c	1.463c
Wheat–**fallow**–rye–cotton	10.273a	10.543a	1.788ab	1.375bc	3.173a	0.478c	12.060b	11.918b	16.773c	20.591b	1.391c	1.741c
Pasture	4.454c	6.420b	1.268bc	1.036c	1.604b	1.348b	5.723c	7.456c	41.001a	33.199a	7.243a	4.915b

[a] For each property (i.e., G-, Fungi:bacteria ratio), LSDs (P < 0.05) compare all treatments together: system (Continuous cotton vs. integrated cropping-livestock system) and grazing (grazed and non-grazed) at each sampling time. Similar letters indicate no significant differences among these treatments at each sampling time.
[b] Bacterial sum: G+ (a15:0, i15:0, a17:0, i17:0), G− (cy17:0, cy19:0, i13:0 3OH, i17:0 3OH) and actinomycetes (10Me16:0, 10Me17:0).
[c] Fungal sum: 18:1ω9c, 16:1ω5c, 18:2ω6c and 18:3ω6c.
[d] No treatment available as cattle were not part of the continuous cotton system.

all sampling times whereas alkaline phosphatase activity was higher in non-grazed areas of pasture in fall 2006 and summer 2007. In addition, alkaline phosphatase activity was higher in the non-grazed areas of the rotation when sampled under fallow and rye in summer 2004 and fall 2006, respectively. Interaction between grazing and integrated cropping-livestock system was significant for alkaline phosphatase activity in fall 2006 and summer 2006 (Table 2). In general, it is difficult to establish specific trends due to system and grazing effects by looking at each enzyme activity at each sampling time. Thus, PCA plots were used in order to better visualize system and grazing effects on the metabolic capacity or diversity in this soil using 10 enzyme activities of C-cycling (β-glucosidase, α-galactosidase, and β-glucosaminidase), P-cycling (acid phosphatase, alkaline phosphatase, and phosphodiesterase) and S-cycling (arylsulfatase).

The PCA plots for summer 2004 and 2007 showed separation (i.e., three groups) between continuous cotton, pasture and the rotation, which is due to the higher β-glucosaminidase, phosphodiesterase and arylsulfatase activities under pasture, intermediate under the rotation and the lowest under continuous cotton (Fig. 5). In addition, the activities of amidohydrolases involved in N-cycling (amidase, urease, and L-asparaginase) were highest under the rotation followed by pasture and were lowest under continuous cotton. Grazing effects were also demonstrated in the PCA plot for summer 2004 which showed separation of the grazed areas and non-grazed areas of pasture due to higher β-glucosaminidase, alkaline phosphatase, L-asparaginase, phosphodiesterase and arylsulfatase activities in the non-grazed areas. The PCA plot for summer 2007 showed separation of the grazed and non-grazed areas of pasture due to higher β-glucosaminidase, alkaline phosphatase, urease and L-asparaginase activities in the non-grazed areas. Generally, β-glucosidase, α-galactosidase and phosphodiesterase activities were no affected by grazing.

The PCA plot in winter 2006 did not show the distinct separation between the rotation and continuous cotton found in summer (2004 and 2007) despite β-glucosidase, urease, L-aspartase and phosphodiesterase activities showed separation among the management (pasture = rotation > continuous cotton) as well as β-glucosaminidase and α-galactosidase (pasture > rotation > continuous cotton). Grazing effects were only found for pasture in the PCA plot for winter 2006 due to higher activities of most enzymes under the non-grazed areas, except for urease and α-galactosidase activities.
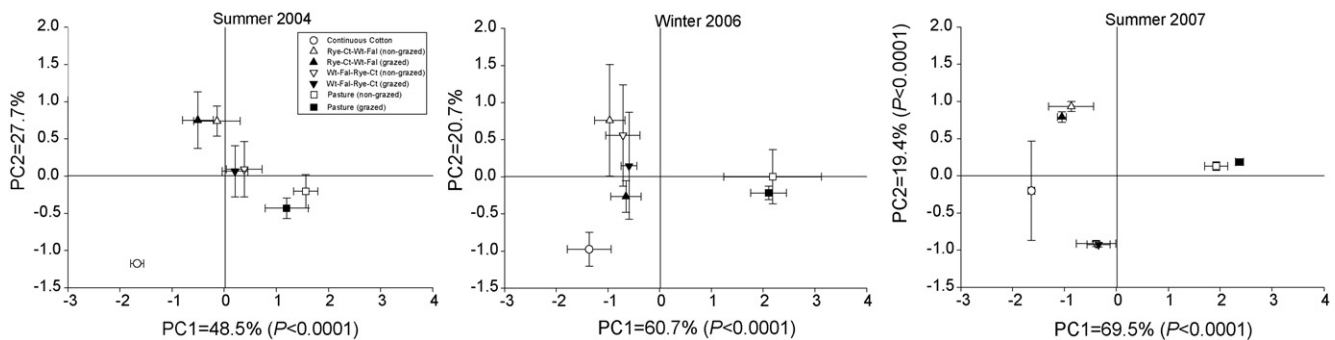

Fig. 3. PCA plots to compare the soil microbial community structure using FAMEs under an integrated cropping-livestock system (grazed and non-grazed areas) and continuous cotton at 0–5 cm depth for different sampling times. A total of 14 FAMEs were used representing fungal indicators (18:1ω9c, 16:1ω5c, 18:2ω6c and 18:3ω6c) and bacterial indicators for G+ (a15:0, i15:0, a17:0, and i17:0), G− (cy17:0, cy19:0, i13:0 3OH, and i17:0 3OH) and actinomycetes (10Me16:0, 10Me17:0). Data shown are means for each system ± SE.
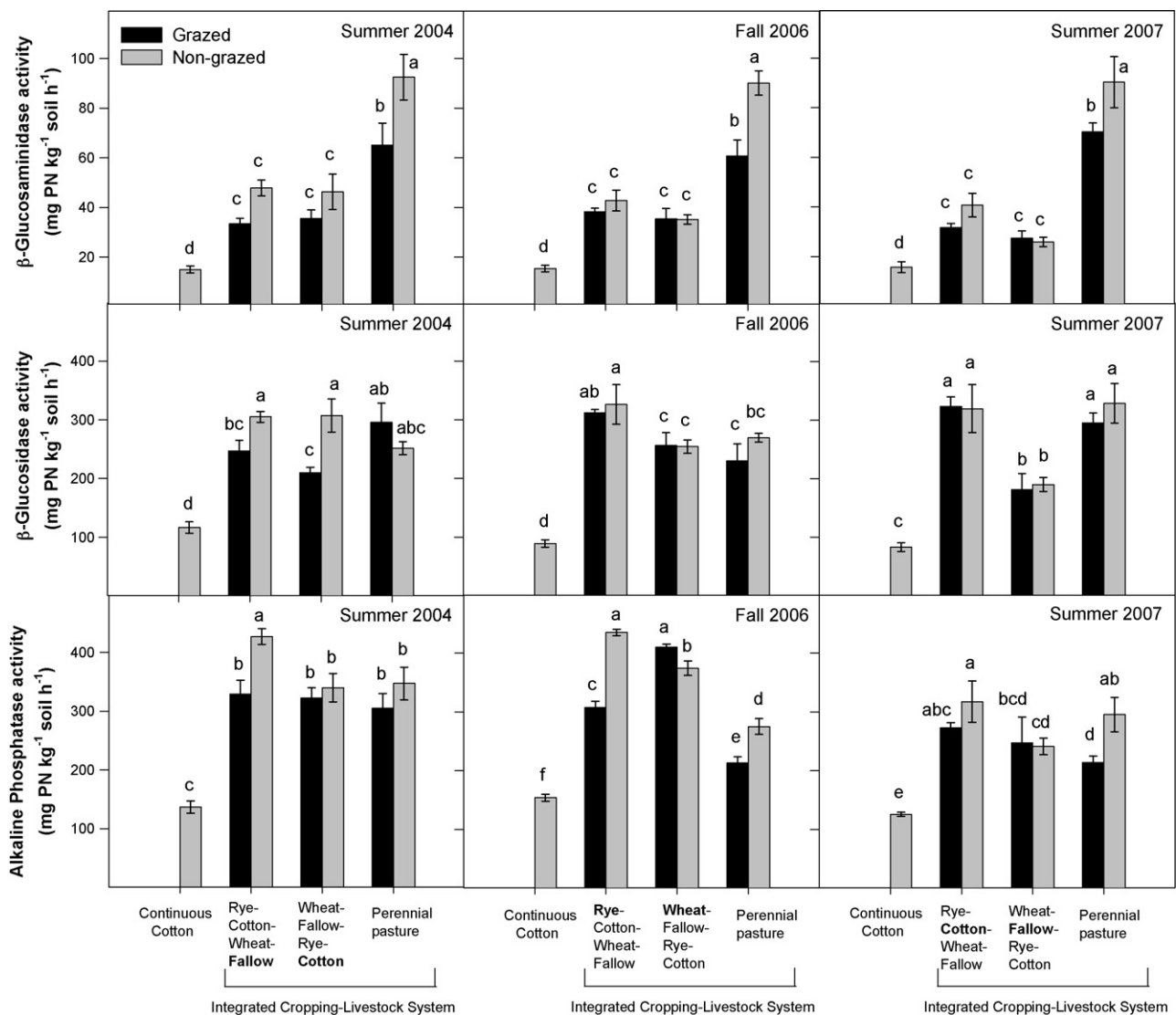
Fig. 4. Selected enzyme activities: (a) β-glucosaminidase, (b) β-glucosidase, and (c) alkaline phosphatase as affected by the system (integrated crop-livestock system vs. continuous cotton) and grazing treatment (grazed vs. non-grazed areas) at 0–5 cm soil depth. Similar letter at the same sampling time indicates no significant treatment differences according to least significant differences (LSD) at $P < 0.05$. Enzyme values are expressed as mean ± S.E.

4. Discussion

4.1. Comparison of soil properties between continuous cotton and integrated cropping-livestock systems

After 5 years at this Texas High Plains research site, Acosta-Martínez et al. (2004) found higher microbial biomass, higher enzyme activities, and shifts in microbial community structure to higher fungal abundances under continuous pasture when compared to continuous cotton; although this trend was not consistent within the crop-rotation paddocks, which only displayed similar patterns while under specific crops. In our study, long-term responses to integrated cropping-livestock system (between 7 and
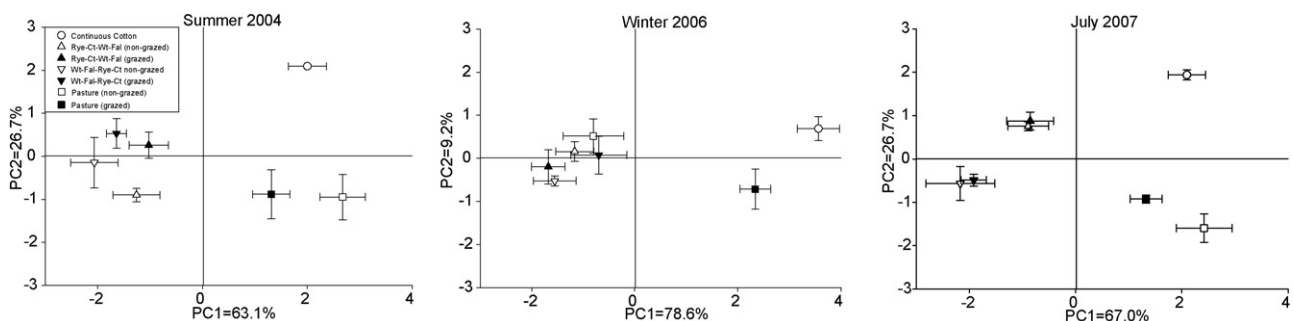

Fig. 5. PCA plots to compare the soil metabolic capacity or diversity using several enzyme activities under an integrated cropping-livestock system (grazed and non-grazed areas) and continuous cotton at 0–5 cm depth for different sampling times. The enzyme activities are involved in C-cycling (β-glucosidase, α-galactosidase, and β-glucosaminidase), N-cycling (β-glucosaminidase, amidase, urease, and ʟ-asparaginase), P-cycling (acid phosphatase, alkaline phosphatase, and phosphodiesterase) and S-cycling (arylsulfatase).

10 years) at this Texas High Plains research site detected higher soil MBC in the pasture and rotation paddocks (regardless of the crop) when compared to continuous cotton. Increased soil microbial biomass (MBC) has been linked to increased soil quality and C sequestration under agriculture systems focusing on minimizing fallow periods while increasing soil organic matter levels by planting perennial grasses or small grain crops (Karlen et al., 1999; Moore et al., 2000; Acosta-Martínez et al., 2004; Sotomayor-Ramirez et al., 2009). Cotton produces far less residue per hectare than other major crops (Lal, 2004) and what little residue C is returned to the soil is potentially lost as a result of intensive tillage practices and fallow periods (Calderón et al., 2001). Thus, it is not surprising to find the higher levels of soil MBC in the integrated cropping-livestock system when compared to continuous cotton. However, our study indicates that consistent increases in soil MBC occur more slowly (>5 years) in the rotation within this integrated cropping-livestock system compared to cotton monoculture practices.

Crop rotations have been recognized for their positive effects on soil properties related to the higher C inputs and diversity of plant residues returned to soils in comparison to continuous systems (Miller and Dick, 1995; Entry et al., 1996; Friedel et al., 1996; Robinson et al., 1996; Moore et al., 2000). After the first 5 years at this Texas High Plains research site, total C was significantly higher under pasture when compared to continuous cotton, and this pattern was not observed in the crop rotation (Acosta-Martínez et al., 2004). However, at the end of 10 years, total C was higher in both the rotation and pasture of the integrated cropping-livestock system (average across grazing treatments: 17.3 g kg^{-1} soil) compared to continuous cotton (11.4 g C kg^{-1} soil). This may suggest that the summer fallow periods limited soil C accumulation at earlier stages of this study despite the higher incorporation of residues and root secretions under the rotation when wheat and/or rye are integrated into cotton cropping practices. Despite the lag time of soil microbial responses with a change from continuous cotton to the rotation evaluated, our study demonstrated that long-term agriculture shifts to an integrated cotton-livestock system composed of a rotation and pasture paddocks as a whole has more potential to increase organic matter content and thus, C sequestration in semi-arid regions of the Texas High Plains.

Our findings of the soil microbial community structure with the tilled continuous cotton system agree with previous studies in which higher bacterial populations have been reported for low biomass return systems due to monoculture and soil disturbance with conventional tillage, which is known to disrupt fungal hyphae (Coleman et al., 1983; Calderón et al., 2001; Kennedy and Schillinger, 2006). Likewise, our findings regarding pasture are in agreement with previous reports showing higher fungal populations in systems associated with increased vegetative and litter cover exclusive of tillage (Frey et al., 1999; Schutter et al., 2001). The rotation showed intermediate bacterial abundances among systems (continuous cotton > rotation > pasture) along with slightly higher fungi:bacterial ratios probably due to the inclusion of other crops in rotations with cotton and the elimination of tillage. Fungal populations were still not significantly different between the crop rotation and continuous cotton after 10 years. This may suggest that shifts in soil fungal abundances may require more than 10 years due to the fallow periods used in this rotation as microbial biomass was already significantly higher in the rotation than continuous cotton. We also should consider that the shifts in soil microbial community structure under the integrated cropping-livestock system can be due to both variations in abundance and distribution among microbial groups and microbial diversity changes at fungi and/or bacteria species level not reflected with FAME analysis. For example, although continuous cotton showed higher bacterial populations than the integrated cropping-livestock system,

bacterial diversity assessment with pyrosequencing demonstrated differences among these systems (i.e., higher Proteobacteria in the integrated cropping-livestock system compared to continuous cotton) (Acosta-Martínez et al., 2010). Overall, increases in fungal:bacterial ratios under this integrated cropping-livestock system compared to continuous cotton can represent potential changes in soil quality and C sequestration as fungal populations tend to have higher C assimilation efficiencies than bacteria populations and store higher amounts of the C they metabolize (Bailey et al., 2002).

Changes in soil microbial communities can influence the potential of soils for enzyme-mediated substrate catalysis (Kandeler et al., 1996). However, there is lack of understanding as to how shifts in microbial community structure reflect changes in soil enzyme activities. For example, we found higher enzyme activities positively correlating with the higher fungal abundances in pasture when compared to continuous cotton (Miller et al., 1998; Parham and Deng, 2000; Acosta-Martínez et al., 2004). On the other hand, while enzyme activities were higher in the crop rotation when compared to continuous cotton, fungal abundances did not differ significantly from continuous cotton according to the fungal FAME markers. PCA plots demonstrated a distinct microbial community separation between the rotation and continuous cotton with several FAMEs. This may suggest more complex soil microbial interactions that involve extracellular sources of enzyme activity. In addition, other studies in the same integrated cropping-livestock system found positive correlations between certain bacteria (Proteobacteria, Firmicutes, Chloroflexi, Verrucomicrobiae and Fibrobacteres) and alkaline phosphatase and β-glucosidase activities when compared to continuous cotton (Acosta-Martínez et al., 2010), suggesting that shifts in bacterial diversity within the integrated cropping livestock system play a critical role in nutrient cycling as a potential source of soil enzymes.

Certain enzymes including β-glucosaminidase, which is involved in N-cycling (i.e., chitin degradation), have been recognized to be primarily produced by fungi (Parham and Deng, 2000). Other studies found a positive correlation between β-glucosaminidase activity and Proteobacteria only, but not with any other bacteria found in this soil under these same systems, suggesting that β-glucosaminidase is mainly produced by fungi populations (Acosta-Martínez et al., 2010). These findings may indicate that the higher β-glucosaminidase activity in the rotation could anticipate that fungal populations may be different between the rotation and continuous cotton in terms of abundance, distribution and/or diversity not detected by FAME fungal markers.

Although enzyme activities were higher under the integrated cropping-livestock system compared to continuous cotton, the levels of the enzyme activities differed within the integrated cropping-livestock system for pasture vs. the rotation depending on the enzyme. For example, there were generally similar levels of the activities of β-glucosidase (involved in cellulose degradation) or alkaline phosphatase (involved in P transformation) in soil under the rotation and pasture whereas β-glucosaminidase activity (chitin degradation) was higher under pasture than under the rotation. These trends for β-glucosaminidase activity may be influenced by the differences in microbial community structure as shown by the PCAs plots (i.e., higher fungal populations under pasture than the rotation) among the systems, because this enzyme has been shown to be mainly produced by fungal populations. These trends may also indicate differences in the substrates available in soil under different management. For example, the activities of amidohydrolases also involved in N-cycling (i.e., amidase, urease and asparaginase) were higher under the rotation compared to pasture possibly due to differences in fertilizer application and substrates utilization between pasture and the rotation.

4.2. Grazing effects within the integrated cropping-livestock system

Previous studies have shown that soil microbial biomass responses to livestock grazing is highly variable (Bardgett and Wardle, 2003; Wang et al., 2006; Ingram et al., 2008). Some studies have reported either low or no grazing effects on microbial biomass (Harrison and Bardgett, 2004; Sakaran and Augustine, 2004; Tracy and Frank, 1998). Wang et al. (2006) reported increased soil microbial biomass under cattle grazing bahiagrass (*Paspalum notatum* Flugge) in a sub-tropical pasture, which were attributed to livestock incorporation of surface litter into the soil and root exudation of labile C compounds, stimulating rhizosphere-microbial community abundances (Holland et al., 1996; Yeates et al., 1997; Bardgett et al., 1998; Hamilton and Frank, 2001). Our findings were more in agreement with Ingram et al. (2008), who reported lower microbial biomass in loamy soils under grazed systems at 0–5 cm in a semi-arid region, which were attributed to lower amounts of high quality substrates and soil moisture content compared to lightly grazed areas. Lower microbial biomass and enzyme activities (i.e., β-glucosaminidase, alkaline phosphatase, L-asparaginase, phosphodiestersae and arylsulfatase) under grazed areas compared to the non-grazed areas within the integrated cropping-livestock system may suggest reductions in plant biomass, soil nutrients and soil moisture for microbial communities in this soil. Grazing effects were not consistently detected in all sample periods. This may be due to the lack of heavy grazing in these systems, as cattle alternated between pasture and the rotations during the year (January–July). Other studies have focused in grass systems where grazing management history leads to vegetation succession (Bardgett et al., 2001); while in our study, crops used in the rotation were always the same and the pasture was always under a grass monoculture for 10 years.

The PCA plots using several FAMEs showed grazing effects on the soil microbial community structure of pasture due to higher G− bacteria and lower actinomycetes in the grazed areas. The individual evaluation of FAMEs showed higher abundance of the FAME indicators for actinomycetes under grazed areas for the rotation in some of the samplings (i.e., Table 3). These trends were in agreement with Patra et al. (2005) who reported higher concentration of bacterial phospholipid fatty acids (PLFA) under intensively grazed grassland systems compared to lightly grazed counterparts. Bardgett et al. (2001) summarized that bacterial based energy channels of decomposition dominate communities of heavily grazed sites in grasslands, whereas fungi can facilitate decomposition more successfully in systems that are either slightly grazed or non-grazed. Together with these changes in microbial community structure due to grazing, which suggest higher fungal populations in the non-grazed soil, the higher enzyme activities confirm the significant role of fungal populations in these nutrient cycling enzymatic reactions in the non-grazed soil.

This study suggests that overall, soil enzyme activities involved in subsequent nutrient cycling respond to grazing practices. Higher phosphatases activities in non-grazed areas of pasture and the rotation may reflect changes in soil P-cycling due to grazing. Similar trends for β-glucosaminidase activity could have important implications for both C- and N-cycling dynamics because this enzyme has been recently correlated with N mineralization as well (Ekenler and Tabatabai, 2002). The higher β-glucosaminidase activity in the non-grazed areas compared to the grazed areas under pasture agrees with N mineralization trends due to grazing reported by Ingram et al. (2008), who stated that decreases in N mineralization in heavily grazed systems may be explained in part by soil C and N losses. Enzyme activities not affected by grazing were for example β-glucosidase and α-galactosidase activities, which are involved, respectively, in the last step of cellulose degradation (dissacharide

cellobiose) and the hydrolysis of terminal alpha-galactosyl moieties from glycolipids and glycoproteins. These findings may indicate that the hydrolysis of these substrates is not limited by grazing. In general, despite the lower activities of certain enzymes observed under grazed soil compared to the non-grazed soil, the integration of livestock and cropping production as a system provides an overall increase in the metabolic capacity of soil compared to the continuous cotton system.

5. Conclusions

Our study complemented different research activities evaluating the sustainability of an integrated cropping-livestock system composed of perennial pasture and a rotation for a semi-arid region with limited water availability for agricultural activities. Our previous studies found reductions in supplemental irrigation (by 23%) and nitrogen fertilizer (by 40%) in the integrated cropping-livestock system compared to the continuous cotton system. In this study, we found lower microbial biomass and several enzyme activities in the grazed areas compared to non-grazed areas of the integrated cropping-livestock system; possibly due to reduction of plant biomass and soil nutrients, which may represent significant substrate losses for microbial populations with grazing. Nevertheless, clear and consistent differences in microbial community size and structure and enzyme-mediated reactions of biogeochemical cycling were found in the integrated cropping-livestock system compared to continuous cotton after 10 years. According to our 10-year evaluation, the wheat–fallow–rye–cotton rotation took longer than pasture to demonstrate shifts in soil microbial biomass and enzyme activities compared to continuous cotton (i.e., 7 years) independent which crop was sampled; possibly due to the fallow periods included in this rotation. These findings indicate that integrated cropping-livestock systems are viable alternatives for improving soil quality and metabolic function in this semi-arid region to current practices of specialized systems such as cotton monocultures.

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